

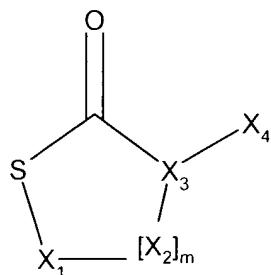
Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (original): A method of generating at least one polydentate metal chelating affinity ligand, which method comprises the steps of

(a) providing at least one scaffold defined by a ring of the general formula (I)



wherein

X₁, X₂ and X₃ irrespective of each other are sp²- or sp³-hybridised carbon atoms or heteroatoms,

X₄ is a nucleophile and

m is an integer of 0-2;

- (b) providing at least one polydentate metal chelating affinity ligand arm on each scaffold by derivatisation of the nucleophilic X_4 groups of said scaffolds while retaining the cyclic structure of the scaffold; and
- (c) opening the ring at a bond between the carbonyl and the sulphur of the derivatised scaffold by adding a reagent that adds one or more metal chelating affinity ligand arms to the scaffold.

Claim 2 (original): The method of claim 1, wherein in formula (I), X_1 , X_2 and X_3 are carbon atoms.

Claim 3 (original): The method of claim 1, wherein in formula (I), X_4 is $-NH_2$.

Claim 4 (original): The method of claim 1, wherein in formula (I), m is 1 and the scaffold is homocysteine thiolactone.

Claim 5 (original): The method of claim 1, wherein in step (b), the derivatisation is provided by adding at least one derivatisation agent comprised of one part, which is electrophilic and hence capable of reacting with X_4 in Formula (I), and a second part, which is a metal chelating affinity ligand.

Claim 6 (original): The method of claim 5, wherein the derivatisation is provided by adding two derivatisation agents, which comprise two different or identical metal chelating functionalities.

Claim 7 (original): The method of claim 5, wherein said at least one derivatisation agent is a halogenated, protected ester.

Claim 8 (original): The method of claim 7, wherein said at least one derivatisation agent is bromo-acetic acid ethyl ester.

Claim 9 (original): The method of claim 1, wherein in step (c), ring-opening is hydrolysis provided by adding a base.

Claim 10 (original): The method of claim 1, wherein the metal chelating affinity ligand arms are protected in step (b).

Claim 11 (original): The method of claim 10, further comprising the steps of deprotecting the chelating affinity ligand arms after step (c) and coupling the resultant compound via its thiol group to a base matrix in order to produce a separation medium.

Claim 12 (original): The method of claim 11, wherein the thiol group is coupled to allyl groups of the base matrix.

Claim 13 (original): The method of claim 11, further comprising a step of allylating the base matrix to provide reactive groups to permit said coupling.

Claim 14 (original): The method of claim 11, wherein the thiol group of the ligand is coupled to the base matrix via an allyl group of allyl glycidyl ether (AGE).

Claim 15 (original): The method of claim 11, further comprising a step of activating the reactive groups of the base matrix.

Claim 16 (original): The method of claim 15, wherein the activating is performed by bromination.

Claim 17 (original): A polydentate metal chelating affinity ligand, or a separation medium comprising a plurality of polydentate metal chelating affinity ligands coupled to a base matrix generated by the method of claim 1.

Claim 18 (original): The ligand or separation medium of claim 17, wherein the ligand is a tridentate ligand.

Claim 19 (withdrawn): A kit which comprises the scaffold of claim 1, which kit includes said scaffold in a solid state together with written instructions for use thereof in the manufacture of a separation medium including a plurality of polydentate metal chelating affinity ligands coupled to a base matrix.

Claim 20 (withdrawn): The kit of claim 19, wherein the scaffold is homocysteine thiolactone.

Claim 21 (original): A separation medium comprising polydentate metal chelating affinity ligands coupled to a base matrix and defined by the general formula base matrix–
 $O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2S-(CH_2)_n-CH(COOH)-N(CH_2COO^-)_2Ni^{2+}$
wherein n is an integer of 2-4.

Claim 22 (original): The medium of claim 21, wherein n = 2.

Claim 23 (withdrawn): A chromatography column for immobilised metal ion adsorption chromatography (IMAC), packed with the separation medium of claim 17.

Claim 24 (withdrawn): A process of separating a target substance from a liquid, which process comprises providing the separation medium of claim 17 to charge said medium with suitable metal ions to form chelates and to contact said medium with the liquid to adsorb the target substance thereon.

Claim 25 (withdrawn): The process of claim 24, further comprising a step of eluting the target substance from the separation medium by adding a liquid that desorbs the target compound from the separation medium.

Claim 26 (withdrawn): The process of claim 25, wherein the eluting is obtained by use of a liquid that comprises a decreasing pH gradient or by applying a gradient of increasing imidazole concentration.